together have caused the majority of TSLS episodes. It is unlikely that these alleles have had a long association with S. pyogenes clones. A fourth allele (speA4) also is present in a single phylogenetic lineage and is 9% divergent from the other three toxin alleles. An absence of synonomous (silent) nucleotide changes in speA2 and speA3 is unusual and suggests that the allelic variation is not selectively neutral, which implies that the toxins are not functionally equivalent. These results may be important in helping to understand the recent increase in frequency and severity of disease caused by S. pyogenes.

L14 ANSWER 23 OF 24 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1991:535204 BIOSIS
DOCUMENT NUMBER: BR41:124939
TITLE: **STREPTOCOCCAL** PYROGENIC EXOTOXIN A **SPEA** AND STREPTOLYSIN O SLO ENHANCE PMNL BINDING TO PROTEIN MATRICES.
AUTHOR(S): BRYANT A; STEVENS D; HACKETT S; SCHLIEVERT P; ZIMMERMAN G
CORPORATE SOURCE: VAMC BOISE, IDAHO.
SOURCE: THIRTY-FIRST INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, CHICAGO, ILLINOIS, USA, SEPTEMBER 29-OCTOBER 2, 1991. PROGRAM ABSTR, (1991) 31 (0), 229.
CODEN: POCHES.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L14 ANSWER 24 OF 24 CONFSCI COPYRIGHT 2000 CSA
ACCESSION NUMBER: 94:51079 CONFSCI
DOCUMENT NUMBER: 94-063049
TITLE: **Mutational** analysis of **streptococcal** pyrogenic exotoxin A (**SpeA**)
AUTHOR: Kline, J.B.; Collins, C.M.
CORPORATE SOURCE: Univ. Miami Sch. Med., Miami, FL, USA
SOURCE: American Society for Microbiology, 1325 Massachusetts Ave., NW, Washington, DC 20005, Abstracts. Paper No. B194.
Meeting Info.: 942 5004: 94th Annual Meeting of the American Society for Microbiology (9425004). Las Vegas, NV (USA). 23-27 May 1994. American Association for Microbiology.
DOCUMENT TYPE: Conference
FILE SEGMENT: DCCP
LANGUAGE: English

FILE 'REGISTRY' ENTERED AT 15:56:03 ON 05 JAN 2000
Searcher : Shears 308-4994

E ASPARAGINE 20/CN

FILE 'CAPLUS' ENTERED AT 15:56:24 ON 05 JAN 2000
L15 41 SEA ABB=ON PLU=ON (ASPARAGINE OR ASN)(W)20 OR ASN20
L16 1 SEA ABB=ON PLU=ON L15 AND (L2 OR L9)
L17 116 SEA ABB=ON PLU=ON (CYSTEINE OR CYS)(W)98 OR CYS98 OR (LYSINE OR LYS)(W)157 OR LYS157 OR (ASPARTIC OR ASP)(1W)45 OR ASP45
L18 0 SEA ABB=ON PLU=ON L17 AND (L2 OR L9)
L19 0 SEA ABB=ON PLU=ON L16 NOT (L5 OR L11)

FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS' ENTERED AT 15:59:36 ON 05 JAN 2000
L20 0 SEA ABB=ON PLU=ON L16
L21 0 SEA ABB=ON PLU=ON L18

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS' ENTERED AT 16:02:42 ON 05 JAN 2000)
L22 1177 SEA ABB=ON PLU=ON SCHLIEVERT P?/AU
L23 58 SEA ABB=ON PLU=ON ROGGIANI M?/AU
L24 716 SEA ABB=ON PLU=ON (STOEHR AUGE J? OR STOEHR J? OR AUGE J?)/AU
L25 539 SEA ABB=ON PLU=ON OHLENDORF D?/AU
L26 5 SEA ABB=ON PLU=ON L22 AND L23 AND L24 AND L25
L27 131 SEA ABB=ON PLU=ON L22 AND (L23 OR L24 OR L25)
L28 16 SEA ABB=ON PLU=ON L23 AND (L24 OR L25)
L29 5 SEA ABB=ON PLU=ON L24 AND L25
L30 2338 SEA ABB=ON PLU=ON L22 OR L23 OR L24 OR L25
L31 140 SEA ABB=ON PLU=ON (L27 OR L30) AND (L2 OR L9)
L32 22 SEA ABB=ON PLU=ON L31 AND (MUTAT? OR MUTANT OR MUTAGEN? OR SUBSTIT? OR POLYMORPH? OR POLY MORPH?)
L33 28 SEA ABB=ON PLU=ON L26 OR L28 OR L29 OR L32
L34 14 DUP REM L33 (14 DUPLICATES REMOVED)

Author (S)

L34 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1
ACCESSION NUMBER: 1998:398421 CAPLUS
DOCUMENT NUMBER: 129:62946
TITLE: **Mutants** of streptococcal exotoxin A and and their use to treat streptococcal toxic shock syndrome
INVENTOR(S): **Schlievert, Patrick M.; Roggiani, Manuela; Stoehr, Jennifer; Ohlendorf, Douglas**
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA; Schlievert, Patrick M.; Roggiani, Manuela; Stoehr, Jennifer; Ohlendorf, Douglas
SOURCE: PCT Int. Appl., 95 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent

Searcher : Shears 308-4994

Lee, L.
09/308830
pt. 1-2

-key terms

FILE 'REGISTRY' ENTERED AT 15:45:13 ON 05 JAN 2000
E STREPTOCOCCAL TOXIN/CN
E SPE A TOXIN/CN
E STREPTOCOCCAL A TOXIN/CN

FILE 'CAPLUS' ENTERED AT 15:45:54 ON 05 JAN 2000
L1 411 SEA ABB=ON PLU=ON (SPE(W)A OR STREPTOCOCC?)(5A)TOXIN
L2 145 SEA ABB=ON PLU=ON (SPE(W)A OR STREPTOCOCC?(3A)A)(5A)TOX IN
L3 15 SEA ABB=ON PLU=ON L2 AND (MUTAT? OR MUTAGEN? OR MUTANT OR POLYMORPH? OR POLY MORPH?)
L4 2 SEA ABB=ON PLU=ON L3 AND SUBSTIT?
L5 15 SEA ABB=ON PLU=ON L3 OR L4

should read "S L2 and substit?"
See pt. 2-2

L5 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:702287 CAPLUS
TITLE: Studies on the Structure and Mechanism of a Bacterial Protein Toxin by Analytical Ultracentrifugation and Small-angle Neutron Scattering
AUTHOR(S): Gilbert, Robert J. C.; Heenan, Richard K.; Timmins, Peter A.; Gingles, Neill A.; Mitchell, Timothy J.; Rowe, Arthur J.; Rossjohn, Jamie; Parker, Michael W.; Andrew, Peter W.; Byron, Olwyn
CORPORATE SOURCE: Department of Biochemistry, University of Leicester, Leicester, LE1 7RH, UK
SOURCE: J. Mol. Biol. (1999), 293(5), 1145-1160
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Pneumolysin, an important virulence factor of the human pathogen **Streptococcus** pneumoniae, is **a** pore-forming **toxin** which also possesses the ability to activate the complement system directly. Pneumolysin binds to cholesterol in cell membrane surfaces as a prelude to pore formation, which involves the oligomerization of the protein. Two important aspects of the pore-forming activity of pneumolysin are therefore the effect of the toxin on bilayer membrane structure and the nature of the self-assocn. into oligomers undergone by it. We have used anal. ultracentrifugation (AUC) to investigate oligomerization and small-angle neutron scattering (SANS) to investigate the changes in membrane structure accompanying pore formation. Pneumolysin self-assocs. in soln. to form oligomeric structures apparently similar to those which appear on the membrane coincident with pore formation. It has previously been demonstrated by us using site-specific chem. derivatization of the protein that the self-interaction preceding oligomerization involves its C-terminal

domain. The AUC expts. described here involved pneumolysin toxoids harbouring **mutations** in different domains, and support our previous conclusions that self-interaction via the C-terminal domain leads to oligomerization and that this may be related to the mechanism by which pneumolysin activates the complement system.SANS data at a variety of neutron contrasts were obtained from liposomes used as model cell membranes in the absence of pneumolysin, and following the addn. of toxin at a no. of concns. These expts. were designed to allow visualization of the effect that pneumolysin has on bilayer membrane structure resulting from oligomerization into a pore-forming complex. The structure of the liposomal membrane alone and following addn. of pneumolysin was calcd. by the fitting of scattering equations directly to the scattering curves. The fitting equations describe scattering from simple three-dimensional scattering vol. models for the structures present in the sample, whose dimensions were varied iteratively within the fitting program. The overall trend was a thinning of the liposome surface on toxin attack, which was countered by the formation of localized structures thicker than the liposome bilayer itself, in a manner dependent on pneumolysin concn. At the neutron contrast match point of the liposomes, pneumolysin oligomers were obsd. Inactive toxin appeared to bind to the liposome but not to cause membrane alteration; subsequent activation of pneumolysin in situbrought about changes in liposome structure similar to those seen in the presence of active toxin. We propose that the changes in membrane structure on toxin attack which we have obsd. are related to the mechanism by which pneumolysin forms pores and provide an important perspective on protein/membrane interactions in general. We discuss these results in the light of published data concerning the interaction of gramicidin with bilayers and the hydrophobic mismatch effect. (c) 1999 Academic Press.

L5 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:486599 CAPLUS
DOCUMENT NUMBER: 131:255683
TITLE: Pneumolysin, **a** protein **toxin** of **Streptococcus** pneumoniae, induces nitric oxide production from macrophages
AUTHOR(S): Braun, Johann S.; Novak, Rodger; Gao, Geli; Murray, Peter J.; Shenep, Jerry L.
CORPORATE SOURCE: Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, 38105, USA
SOURCE: Infect. Immun. (1999), 67(8), 3750-3756
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Nitric oxide (NO) prodn. by inducible NO synthase (iNOS) during

inflammation is an essential element of antimicrobial immunity but can also contribute to host-induced tissue damage. Under conditions of bacterial sepsis, large amts. of NO are produced, causing hypotension, a crit. pathol. feature of septic shock. In sepsis caused by gram-pos. organisms, the bacterial factors contributing to host NO prodn. are poorly characterized. The authors show that **a** sol. **toxin** of **Streptococcus** pneumoniae, pneumolysin (Pln), is a key component initiating NO prodn. from macrophages. In contrast to wild-type bacteria, a **mutant** of S. pneumoniae lacking Pln failed to elicit NO prodn. from murine macrophages. Purified recombinant Pln induced NO prodn. at low concns. and independently of exogenous gamma interferon (IFN-.gamma.) priming of RAW 264.7 macrophages. However, IFN-.gamma. was essential for Pln-induced NO prodn., since primary macrophages from mice lacking the IFN-.gamma. receptor or interferon regulatory factor 1, a transcription factor essential for iNOS expression, failed to produce NO when stimulated with Pln. In addn., Pln acts as an agonist of tumor necrosis factor alpha and interleukin 6 prodn. in macrophages. The properties of Pln, previously identified as a pore-forming hemolysin, also include a role as a general inflammatory agonist.

L5 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:398421 CAPLUS
DOCUMENT NUMBER: 129:62946
TITLE: **Mutants** of streptococcal exotoxin A and and their use to treat streptococcal toxic shock syndrome
INVENTOR(S): Schlievert, Patrick M.; Roggiani, Manuela; Stoehr, Jennifer; Ohlendorf, Douglas
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA; Schlievert, Patrick M.; Roggiani, Manuela; Stoehr, Jennifer; Ohlendorf, Douglas
SOURCE: PCT Int. Appl., 95 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9824911	A2	19980611	WO 1997-US22228	19971205

W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9876257	A1	19980629	AU 1998-76257	19971205
EP 948624	A2	19991013	EP 1997-949752	19971205

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

PRIORITY APPLN. INFO.:	US 1996-32930	19961206
	WO 1997-US22228	19971205

AB This invention is directed to **mutant streptococcal** exotoxin **A** (**SPE-A**) **toxins** or fragments thereof, vaccine and pharmaceutical compns., and methods of using the vaccine and pharmaceutical compns. The preferred **SPE-A toxin** has at least one amino acid change and is substantially non-lethal compared with the wild type **SPE-A toxin**. The **mutant** **SPE-A toxins** can form vaccine compns. useful to protect animals against the biol. activities of wild type **SPE-A toxin**. The esp. preferred **mutants** for vaccine compns. are **mutant SPE -A toxins** that immunoreact with polyclonal neutralizing antibodies to wild-type **SPE-A toxin**, are nontoxic, and optionally have a decrease in potentiation of endotoxin shock and a decrease in T-cell mitogenicity. The esp. preferred **mutants** have a change in the Asn-20 residue (e.g., N20D with an aspartic acid **substituted** for Asn-20 in the mature toxin). In addn., changes at amino acid 98 that result in lack of a cysteine group at that location (C98S) also result in a **mutant** toxin that a decrease in enhancement in endotoxin shock and a 4-fold decrease in mitogenicity. The K157E **mutant** is nonlethal but retains mitogenicity comparable to the wild-type **SPE-A toxin**. The triple **mutant** N20D/D45N/C98S has no detectable toxicity in vivo and is safe and effective in protecting animals in a streptococcal toxic shock syndrome model.

L5 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER:	1998:249982 CAPLUS
DOCUMENT NUMBER:	129:14256
TITLE:	High-frequency intracellular infection and erythrogenic toxin A expression undergo phase variation in M1 group A streptococci
AUTHOR(S):	Cleary, P. Patrick; Mclandsborough, Lynne; Ikeda, Leo; Cue, David; Krawczak, Jim; Lam, Hong
CORPORATE SOURCE:	Department of Microbiology, University of Minnesota, Minneapolis, MN, 55126, USA
SOURCE:	Mol. Microbiol. (1998), 28(1), 157-167 CODEN: MOMIEE; ISSN: 0950-382X
PUBLISHER:	Blackwell Science Ltd.

DOCUMENT TYPE: Journal
LANGUAGE: English
AB A clonal variant of serotype M1 group A streptococcus, strain 90-131, disseminated to several continents, where it was assocd. with severe systemic infections and toxic shock. Although this strain harbors the speA gene and is efficiently internalized by human epithelial cells, clin. isolates often fail to express the erythrogenic toxin under lab. growth conditions. Cultures of strain 90-131 were obsd. to phase vary between small, dry, compact and larger, more mucoid colonies. The former were shown to be poorly internalized by epithelial cells. Anal. of RNA by Northern hybridization demonstrated that the emm1, hasA and speA genes were weakly transcribed in cultures derived from the small colonies and highly transcribed in those derived from the large colonies. An insertion **mutation** in mga (the multigene activator) downregulated the invasion of epithelial cells and the transcription of emm1 and hasA, but had little impact on the transcription of speA. These are the first data to suggest the existence of a common regulatory circuit linking intracellular invasion, M protein, hyaluronic acid capsule and erythrogenic **toxin** expression by group **A streptococcus**. Moreover, the genetic instability of toxin expression exhibited by this serotype may impact on lab. studies that attempt to assoc. toxin prodn. with toxic shock.

L5 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:214153 CAPLUS
DOCUMENT NUMBER: 128:319257
TITLE: Reduced virulence of group A streptococcal Tn916 **mutants** that do not produce streptolysin S
AUTHOR(S): Betschel, Stephen D.; Borgia, Sergio M.; Barg, Neil L.; Low, Donald E.; De Azavedo, Joyce C. S.
CORPORATE SOURCE: Department of Microbiology, Mount Sinai and Princess Margaret Hospitals, Toronto, ON, M5G 1X5, Can.
SOURCE: Infect. Immun. (1998), 66(4), 1671-1679
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Streptolysin S (SLS) is a potent cytolytic **toxin** produced by nearly all group **A streptococci** (GAS). SLS-deficient Tn916 insertional **mutants** were generated from two clin. isolates of GAS, MGAS166s and T18Ps (M serotypes 1 and 18, resp.), by transposon **mutagenesis** using Tn916 donor strain Enterococcus faecalis CG110. Representative nonhemolytic transconjugants SBNH5 and SB30-2 each harbored a single Tn916 insertion in identical loci. The insertion in SBNH5 was

located in the promoter region of an open reading frame, designated sagA, rendering it transcriptionally inactive. Protease, streptolysin O, and DNase activities and the prodn. of M protein remained the same in the nonhemolytic **mutants** and the wild-type strains, as did the growth rates and exoprotein profiles. Transconjugants were evaluated in an established murine model by injecting the organisms s.c. and monitoring the mice for alterations in wt. and the development of necrotic lesions. Animals infected with SBNH5, compared to those infected with MGAS166s, gained wt. during the first 24 h (+1.15 vs. -1.16 g; $P < 0.05$) and had fewer necrotic lesions (0 vs. 7; $P = 0.0007$). Animals infected with SB30-2, compared to those infected with T18Ps, also gained wt. within the first 24 h (+0.54 vs. -0.66 g; $P < 0.05$) and produced fewer necrotic lesions (1 vs. 8; $P = 0.001$). Revertants of the **mutants** in which Tn916 had been excised regained the hemolytic phenotype and the virulence profile of the wild-type strains. This study demonstrates that SLS-deficient **mutants** of GAS, belonging to different M serotypes and contg. identical Tn916 **mutations**, are markedly less virulent than their isogenic parents.

L5 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:121424 CAPLUS
DOCUMENT NUMBER: 126:128161
TITLE: **Mutants** of **streptococcal toxin A** and methods of use as vaccine
INVENTOR(S): Schlievert, Patrick M.; Roggiani, Manuela; Stoehr, Jennifer; Ohlendorf, Douglas
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA; Schlievert, Patrick M.; Roggiani, Manuela; Stoehr, Jennifer; Ohlendorf, Douglas
SOURCE: PCT Int. Appl., 100 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9640930	A1	19961219	WO 1996-US10252	19960607
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA				
CA 2221480	AA	19961219	CA 1996-2221480	19960607

Searcher : Shears 308-4994

AU 9662782	A1	19961230	AU 1996-62782	19960607
EP 832241	A1	19980401	EP 1996-921589	19960607
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
JP 11514844	T2	19991221	JP 1996-502265	19960607
PRIORITY APPLN. INFO.:			US 1995-480261	19950607
			WO 1996-US10252	19960607

AB This invention is directed to **mutant streptococcal** pyrogenic exotoxin **A** (**SPE-A toxins**; scarlet fever **toxin** A) or fragments thereof, vaccine and pharmaceutical compns., and methods of using the vaccine and pharmaceutical compns. The preferred **SPE-A toxin** has at least one amino acid change and is substantially non-lethal compared with the wild type **SPE-A toxin**. The **mutant SPE-A toxins** can form vaccine compns. useful to protect animals against the biol. activities of wild type **SPE-A toxin**. Prepn. of **SPE-A toxin mutants** having **mutation** at 20-N.fwdarw.D, 20-N.fwdarw.D/157-K.fwdarw.E, 20-N.fwdarw.D/98-C.fwdarw.S by site-specific **mutation**, and biol. activities of the **mutants** were demonstrated.

L5 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER:	1995:474650 CAPLUS
DOCUMENT NUMBER:	122:236678
TITLE:	Staphylococcal and group **A streptococcal** pyrogenic **toxin** superantigens associated with toxic shock syndrome and related illnesses
AUTHOR(S):	Schlievert, P. M.; Leonard, B. A. B.; Lee, P. K.; Kreiswirth, B. N.; Eisner, W.; Projan, S. J.; Novick, R. P.
CORPORATE SOURCE:	Department Microbiology, University Minnesota, Minneapolis, MN, USA
SOURCE:	Zentralbl. Bakteriol., Suppl. (1994), 26 303-11 CODEN: ZBASE2; ISSN: 0941-018X
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Toxic shock syndrome (TSS) and TSS-like illness are acute onset, multisystem diseases assocd. with pyrogenic toxin superantigen-producing Staphylococcus aureus and group A streptococci, resp. Major categories of staphylococcal TSS include menstrual and non-menstrual, with the latter being further subdivided into post surgical, skin infection-assocd., influenza-assocd., and the recently described RED syndrome in AIDS patients. TSS-like illness is primarily assocd. with skin infections by M type 1 and 3 group A streptococci. Several mechanisms have been proposed to explain the hypotension and shock

assocd. with TSS and TSS-like illness, including lymphokine release from T cells, monokine release, endotoxin enhancement, and direct toxin-induced capillary leak. By making use of gene fusion constructs between tst H, which encodes human TSS toxin-1 and tst O, which encodes a biol. inactive ovine TSS toxin variant, data were obtained that localized the hypotensive effects of TSST-1 to the N-terminal half of the toxin. In contrast, the T cell proliferative effect was localized to the C-terminal half. Similarly, by use of site specific **mutagenesis**, it was possible to sep. the lethal activities of streptococcal scarlet fever toxin type A from the T cell proliferative effects. The data indicate the induction of hypotension and shock by these toxins does not depend on their T cell proliferative effects. TSS toxin-1 has been crystd. as an initial step to detg. the 3-dimensional structure.

L5 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1993:664446 CAPLUS
DOCUMENT NUMBER: 119:264446
TITLE: Identification of hydrogen peroxide as a **Streptococcus** pneumoniae **toxin** for rat alveolar epithelial cells
AUTHOR(S): Duane, Peter G.; Rubins, Jeffrey B.; Weisel, Heather R.; Janoff, Edward N.
CORPORATE SOURCE: Dep. Med., Minneapolis Veterans, Minneapolis, MN, 55417, USA
SOURCE: Infect. Immun. (1993), 61(10), 4392-7
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors examd. the effects of S. pneumoniae-assocd. alveolar epithelial cell injury by factors other than S. pneumoniae-derived pneumolysin or phagocyte products by exposing cultured rat type II alveolar epithelial cells (RAEC) to S. pneumoniae **mutants** that lacked pneumolysin activity. The authors found that **mutant** pneumolysin-deficient strains of S. pneumoniae produced injury to RAEC similar to that produced by the parent strains. A toxin of type 14 S. pneumoniae was distinguished from pneumolysin by physicochem. (i.e., mol. mass and heat stability) and functional (i.e., hemolytic activity and cytotoxic activity) properties and was identified as hydrogen peroxide. All S. pneumoniae strains tested produced hydrogen peroxide, and in many strains hydrogen peroxide prodn. was comparable to that of activated neutrophils. The authors conclude that S. pneumoniae produces hydrogen peroxide in concns. that are cytotoxic to RAEC in vitro and that alveolar epithelial damage due to hydrogen peroxide may be involved in the pathogenesis of host cellular injury in pneumococcal pneumonia.

L5 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1993:647528 CAPLUS
DOCUMENT NUMBER: 119:247528
TITLE: **Mutations** affecting MHC class II binding of the superantigen **streptococcal** erythrogenic **toxin A**
AUTHOR(S): Hartwig, Udo F.; Fleischer, Bernhard
CORPORATE SOURCE: 1st Dep. Med., Univ. Mainz, Mainz, D-6500, Germany
SOURCE: Int. Immunol. (1993), 5(8), 869-75
CODEN: INIMEN; ISSN: 0953-8178
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Streptococcal pyrogenic exotoxin A (SPEA) is an important pathogenicity factor of group A streptococci. It is a member of the family of superantigens produced by Staphylococcus aureus and Streptococcus pyogenes, and its T lymphocyte stimulating activity is involved in the pathogenesis of certain diseases caused by pyogenic streptococci. In this study the authors have generated 9 **mutant** SPEA mols. by **substituting** amino acids in the regions of homol. between different streptococcal and staphylococcal superantigens. An addnl. **mutant** was created by deletion of the 10 N-terminal amino acids. The **mutants** were expressed as fusion proteins. Several **mutations** led to a loss of function due to a loss of class II-binding activity. Such loss **mutations** did not cluster to a certain region of the SPEA mol. Rather, even a **substitution** of neighboring amino acids had opposite effects. None of the loss **mutations** affected the binding of neutralizing mAb and all loss **mutants** could be pptd. in Ouchterlony tests by a polyclonal anti-SPEA serum. Thus, the functional activities of SPEA, and probably of other superantigens as well, cannot be attributed to a defined region of the mol.

L5 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1993:211273 CAPLUS
DOCUMENT NUMBER: 118:211273
TITLE: Staphylococcus aureus toxic shock syndrome **toxin** 1 and **Streptococcus** pyogenes erythrogenic **toxin A** modulate inflammatory mediator release from human neutrophils
AUTHOR(S): Hensler, T.; Koeller, M.; Geoffroy, C.; Alouf, J. E.; Koenig, W.
CORPORATE SOURCE: AG Infektabwehrmech., Ruhr-Univ. Bochum, Bochum, 4630, Germany
SOURCE: Infect. Immun. (1993), 61(3), 1055-61
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal

LANGUAGE: English

AB Influence of staphylococcal toxic shock syndrome **toxin** 1 and **streptococcal** erythrogenic (pyrogenic) **toxin A** (ETA) was studied on intact and digitonin-permeabilized human **polymorphonuclear** granulocytes (PMNs). As shown by reversed-phase HPLC anal., toxic shock syndrome toxin 1 or ETA alone in the absence of any addnl. stimulus, did not induce the generation of the chemoattractant leukotriene B4 (LTB4) from PMNs in a wide range of concns. In addn., pretreatment of intact PMNs with either toxin potentiated formyl-methionyl-leucyl-phenylalanine (fMLP)- and washed S. aureus cell-induced generation of LTB4 in a time- and dose-dependent manner. This increase included LTB4 as well as its inactive .omega.-oxidated compds. Further studies revealed evidence that toxin exposure was accompanied by enhanced cellular receptor expression for fMLP as well as for LTB4. The intrinsic GTPase activity of membrane fractions was modulated by both toxins. Short-term incubation with ETA increased the GTPase activity of PMNs .ltoreq. 141%. Inhibitory effects were obtained when GTP-binding protein functions were stimulated with NaF. In addn., specific binding of Gpp(NH)p to GTP-binding protein was inhibited by both toxins during the first 10 min of incubation and was restored at later times of incubation. Therefore, toxins may have affected the signal transduction pathways of human PMNs, which resulted in immunomodulatory functions.

L5 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1993:209269 CAPLUS

DOCUMENT NUMBER: 118:209269

TITLE: Geographic and temporal distribution and molecular characterization of two highly pathogenic clones of Streptococcus pyogenes expressing allelic variants of pyrogenic exotoxin A (scarlet fever toxin)

AUTHOR(S): Musser, James M.; Kapur, Vivek; Kanjilal, Sagarika; Shah, Uma; Musher, Daniel M.; Barg, Neil L.; Johnston, Kenneth H.; Schlievert, Patrick M.; Henrichsen, Jorgen; et al.

CORPORATE SOURCE: Sect. Mol. Pathobiol., Baylor Coll. Med., Houston, TX, USA

SOURCE: J. Infect. Dis. (1993), 167(2), 337-46
CODEN: JIDIAQ; ISSN: 0022-1899

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The mol. population genetics and pathogenic potential of North American and European invasive strains of S. pyogenes were assessed. Isolates from recent invasive infections and from infections in the 1920s and 1930s were characterized for multilocus enzyme genotype and allelic variation in the gene (speA) that encodes **streptococcal** pyrogenic exotoxin (**SPE**) **A**

(scarlet fever **toxin**). A subset of strains was studied for allelic variation in genes that encode SPE B and streptokinase. All contemporary strains assigned to electrophoretic types (ETs) 1 and 2 that synthesize SPE A have the speA2 and speA3 allelic variants, resp., and their relative virulence in 2 mouse models is similar to that of strains of the same ET and M protein types recovered earlier. In contrast, ET 1 and 2 isolates from disease episodes in the 1920s and 1930s contain the speA1 allele. The data suggest there may be temporal and geog. variation in the occurrence of clone-virulence factor allele combinations, an observation that may in part explain fluctuations in disease frequency, severity, and character.

L5 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1993:17352 CAPLUS
DOCUMENT NUMBER: 118:17352
TITLE: Genetic diversity in T1M1 group A streptococci in relation to clinical outcome of infection
AUTHOR(S): Norgren, Mari; Norrby, Anna; Holm, Stig E.
CORPORATE SOURCE: Dep. CLin. Bacteriol., Univ. Umea, Swed.
SOURCE: J. Infect. Dis. (1992), 166(5), 1014-20
CODEN: JIDIAQ; ISSN: 0022-1899
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Genetic diversity was found at high frequency downstream of the emm1 gene among T1M1 group A streptococci (GAS) isolated in Scandinavia during a recent epidemic. Clonal variation was also seen in the speA and speB genes but at much lower frequency; no variation was detected in the speC gene. Erythrogenic toxin A was expressed at low levels in all strains; erythrogenic toxins B and C were produced in high amts. All strains harbored the speA, speB, and speC genes, regardless of the amt. of toxin produced. No correlation was found between one specific T1M1 clone and the more serious infections when isolates from bacteremic patients (fatalities or survivors), those with uncomplicated infections, and healthy carriers were compared. Similar results were obtained in a family study in which 3 family members were asymptomatic carriers of the same GAS T1M1 clone as in the bacteremic patient, defined by genotypic and phenotypic expts.

L5 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1992:631312 CAPLUS
DOCUMENT NUMBER: 117:231312
TITLE: Streptococcal pyrogenic exotoxin A and streptolysin O enhance **polymorphonuclear** leukocyte binding to gelatin matrixes
AUTHOR(S): Bryant, Amy E.; Kehoe, Michael A.; Stevens, Dennis L.
CORPORATE SOURCE: Infect. Dis. Sect., VA Med. Cent., Boise, ID, 83702, USA

SOURCE: J. Infect. Dis. (1992), 166(1), 165-9
CODEN: JIDIAQ; ISSN: 0022-1899
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Autopsy data from cases of streptococcal toxic shock demonstrate accumulation of **polymorphonuclear** leukocytes (PMNL) within lung and soft tissue microvasculature. Because of the increased prevalence of streptococcal pyrogenic exotoxin A (SPEA)-producing strains assocd. with streptococcal toxic shock syndrome, expts. were done to det. whether SPEA or streptolysin O (SLO, a thiol-activated cytolysin produced by all group A streptococci) could stimulate PMNL-dependent adherence mechanisms in vitro. SPEA (0.01-10 .mu.g/5.5 .times. 106 PMNL) only modestly enhanced PMNL adherence over the entire range of concns. tested. In contrast, SLO-induced PMNL binding was highly dose dependent (maximal binding, 55.1% at 0.5 hemolytic units/5.5 .times. 106 PMNL) and was mediated by CD11/CD18 adherence glycoprotein.

L5 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1989:190637 CAPLUS
DOCUMENT NUMBER: 110:190637

TITLE: Oral administration of **a streptococcal** antigen coupled to cholera **toxin** B subunit evokes strong antibody responses in salivary glands and extramucosal tissues

AUTHOR(S): Czerkinsky, Cecil; Russell, Michael W.; Lycke, Nils; Lindblad, Marianne; Holmgren, Jan
CORPORATE SOURCE: Dep. Med. Microbiol., Univ. Goeteborg, Goeteborg, 41346, Swed.

SOURCE: Infect. Immun. (1989), 57(4), 1072-7
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Generation of local and systemic immune responses by the oral administration of antigens is frequently inefficient, requiring large quantities of immunogens and yielding only modest antibody responses. Oral administration of microgram amts. of Streptococcus **mutants** protein antigen I/II covalently coupled to the B subunit of cholera toxin elicits vigorous mucosal as well as extramucosal IgA and G antistreptococcal antibody responses in mice. These responses were manifested by the presence of large nos. of antibody-secreting cells in salivary glands, mesenteric lymph nodes, and spleens and by the development of high levels of circulating antibodies. This novel immunization strategy may find broad application in the construction of oral vaccines for the control of infectious diseases caused by pathogens encountered at mucosal and extramucosal sites.

L5 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1982:31333 CAPLUS
DOCUMENT NUMBER: 96:31333
TITLE: Phage-host interactions and the production of type A streptococcal exotoxin in group A streptococci
AUTHOR(S): McKane, Larry; Ferretti, Joseph J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA
SOURCE: Infect. Immun. (1981), 34(3), 915-19
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The infection of Streptococcus pyrogenes nontoxigenic strain T253 with bacteriophage T12 to form lysogen T253 (T12) resulted in the prodn. of type **A streptococcal** exotoxin (erythrogenic **toxin** or streptococcal pyrogenic exotoxin). Two lines of evidence indicated that lysogeny per se was not sufficient to promote toxigenic conversion of strain T253. First, a virulent **mutant** of phage T12, unable to form stable lysogens, was able to affect type A exotoxin prodn. by strain T253. An unrelated virulent phage A25 did not affect type A exotoxin prodn. after infection of strain T253. Second, the temperate phage H4489A, which established stable lysogens with strain T253, did not promote type A exotoxin prodn. Apparently, there is a strain specificity to the phage-host interaction which affects type A exotoxin synthesis. Addnl. evidence is presented which indicates that type A streptococcal exotoxin was not a structural component of phage T12.

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS' ENTERED AT 15:49:45 ON 05 JAN 2000)
L6 54 S L5
L7 20 DUP REM L6 (34 DUPLICATES REMOVED)

L7 ANSWER 1 OF 20 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-358008 [30] WPIDS
DOC. NO. CPI: C1999-105956
TITLE: Non-toxic modified staphylococcal enterotoxins.
DERWENT CLASS: B04 D16
INVENTOR(S): BOHACH, G I
PATENT ASSIGNEE(S): (IDAH-N) IDAHO RES FOUND INC
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9927889	A2	19990610	(199930)*	EN	25

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC

MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI
GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK
SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 9916028 A 19990616 (199945)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9927889	A2	WO 1998-US25107	19981201
AU 9916028	A	AU 1999-16028	19981201

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9916028	A	Based on	WO 9927889

PRIORITY APPLN. INFO: US 1997-67357 19971202
AN 1999-358008 [30] WPIDS
AB WO 9927889 A UPAB: 19990802
NOVELTY - Pyrogenic toxins modified in the disulfide loop region are new.

DETAILED DESCRIPTION - A modified pyrogenic toxin derived from a native disulfide loop-containing pyrogenic toxin comprises:

(1) a disulfide loop region containing no more than 10 amino acid residues; or

(2) deletion of at least about 40% of the amino acid residues within the disulfide loop of the native toxin.

INDEPENDENT CLAIMS are also included for:

(1) an isolated nucleotide comprising a nucleotide sequence encoding a modified pyrogenic toxin as in (a) above, or a modified staphylococcal enterotoxin derived from a native staphylococcal enterotoxin having a deletion as in (b) above; and

(2) a modified pyrogenic toxin derived from a native type C staphylococcal enterotoxin, where the modified toxin comprises (a) as above.

ACTIVITY - Cytostatic; Proliferative; Immunostimulant.

MECHANISM OF ACTION - Cytokine Inducer.

USE - The modified toxins are produced to have a reduced toxicity compared to the native toxin. The modified toxins can be used in a similar manner to the native toxins. The staphylococcal enterotoxins are potent activators of T-cells, resulting in proliferation and the generation of cytotoxic T-cells. Staphylococcal enterotoxins, aside from the acute gastroenteritis and toxic shock syndrome associated with them, have a variety of other beneficial biological effects. The biological effects are in

part due to the ability of the enterotoxins to induce cytokines. The antitumor activity of treating cancer in rabbits utilizing 40-60 mu g/kg of a staphylococcal enterotoxin is known (see WO9110680 and WO9324136).

ADVANTAGE - The modified toxin has a substantially decreased toxicity compared to the native toxin. The emetic response or fever inducing activity is decreased by at least about 100-fold in comparison to the native toxin. (All claimed)

It has been previously shown that the minimal emetic dose of wild type SEC for M. nemestrina was 0.1 mu g/kg. For initial experiments in which the emetic ability was tested, loop **mutant** toxins were administered at 10 mu g/kg. This insured an excess of toxin over the wild type SEC1 minimal emetic dose. Following intragastric toxin inhibition, animals were observed for at least 12 hours for an emesis response. SEC1-12AA did not show emesis at the 10 mu g/kg concentration and was subsequently tested for emesis at a higher toxin concentration, 250 mu g/kg. Even at this higher dosage level, the SEC1-12AA loop **mutant** toxin showed no emetic response.

Dwg.0/0

L7 ANSWER 2 OF 20 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999346155 MEDLINE
DOCUMENT NUMBER: 99346155
TITLE: Pneumolysin, **a** protein **toxin** of **Streptococcus** pneumoniae, induces nitric oxide production from macrophages.
AUTHOR: Braun J S; Novak R; Gao G; Murray P J; Shenep J L
CORPORATE SOURCE: Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, USA.. johann.braun@stjude.org
CONTRACT NUMBER: AI 27913 (NIAID)
P30 CA 21765 (NCI)
SOURCE: INFECTION AND IMMUNITY, (1999 Aug) 67 (8) 3750-6.
Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199910
ENTRY WEEK: 19991002

AB Nitric oxide (NO) production by inducible NO synthase (iNOS) during inflammation is an essential element of antimicrobial immunity but can also contribute to host-induced tissue damage. Under conditions of bacterial sepsis, large amounts of NO are produced, causing hypotension, a critical pathological feature of septic shock. In sepsis caused by gram-positive organisms, the bacterial factors contributing to host NO production are poorly characterized. We show that **a** soluble **toxin** of **Streptococcus**

pneumoniae, pneumolysin (Pln), is a key component initiating NO production from macrophages. In contrast to wild-type bacteria, a **mutant** of S. pneumoniae lacking Pln failed to elicit NO production from murine macrophages. Purified recombinant Pln induced NO production at low concentrations and independently of exogenous gamma interferon (IFN-gamma) priming of RAW 264.7 macrophages. However, IFN-gamma was essential for Pln-induced NO production, since primary macrophages from mice lacking the IFN-gamma receptor or interferon regulatory factor 1, a transcription factor essential for iNOS expression, failed to produce NO when stimulated with Pln. In addition, Pln acts as an agonist of tumor necrosis factor alpha and interleukin 6 production in macrophages. The properties of Pln, previously identified as a pore-forming hemolysin, also include a role as a general inflammatory agonist.

L7 ANSWER 3 OF 20 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 1999409832 EMBASE
TITLE: Studies on the structure and mechanism of a bacterial protein toxin by analytical ultracentrifugation and small-angle neutron scattering.
AUTHOR: Gilbert R.J.C.; Heenan R.K.; Timmins P.A.; Gingles N.A.; Mitchell T.J.; Rowe A.J.; Rossjohn J.; Parker M.W.; Andrew P.W.; Byron O.
CORPORATE SOURCE: R.J.C. Gilbert, Division of Structural Biology, Wellcome Trust Ctr. Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, United Kingdom. gilbert@strubi.ox.ac.uk
SOURCE: Journal of Molecular Biology, (12 Nov 1999) 293/5 (1145-1160).
Refs: 69
ISSN: 0022-2836 CODEN: JMOBAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Pneumolysin, an important virulence factor of the human pathogen **Streptococcus** pneumoniae, is **a** pore-forming **toxin** which also possesses the ability to activate the complement system directly. Pneumolysin binds to cholesterol in cell membrane surfaces as a prelude to pore formation, which in involves the oligomerization of the protein. Two important aspects of the pore-forming activity of pneumolysin are therefore the effect of the toxin on bilayer membrane structure and the nature of the self-association into oligomers undergone by it. We have used analytical ultracentrifugation (AUC) to investigate oligomerization and small-angle neutron scattering (SANS) to investigate the changes in membrane structure accompanying pore formation. Pneumolysin

self-associates in solution to form oligomeric structures apparently similar to those which appear on the membrane coincident with pore formation. It has previously been demonstrated by us using site-specific chemical derivatization of the protein that the self-interaction preceding oligomerization involves its C-terminal domain. The AUC experiments described here involved pneumolysin toxoids harbouring **mutations** in different domains, and support our previous conclusions that self-interaction via the C-terminal domain leads to oligomerization and that this may be related to the mechanism by which pneumolysin activates the complement system. SANS data at a variety of neutron contrasts were obtained from liposomes used as model cell membranes in the absence of pneumolysin, and following the addition of toxin at a number of concentrations. These experiments were designed to allow visualization of the effect that pneumolysin has on bilayer membrane structure resulting from oligomerization into a pore-forming complex. The structure of the liposomal membrane alone and following addition of pneumolysin was calculated by the fitting of scattering equations directly to the scattering curves. The fitting equations describe scattering from simple three-dimensional scattering volume models for the structures present in the sample, whose dimensions were varied iteratively within the fitting program. The overall trend was a thinning of the liposome surface on toxin attack, which was countered by the formation of localized structures thicker than the liposome bilayer itself, in a manner dependent on pneumolysin concentration. At the neutron contrast match point of the liposomes, pneumolysin oligomers were observed. Inactive toxin appeared to bind to the liposome but not to cause membrane alteration; subsequent activation of pneumolysin in situ brought about changes in liposome structure similar to those seen in the presence of active toxin. We propose that the changes in membrane structure on toxin attack which we have observed are related to the mechanism by which pneumolysin forms pores and provide an important perspective on protein/membrane interactions in general. We discuss these results in the light of published data concerning the interaction of gramicidin with bilayers and the hydrophobic mismatch effect.

L7 ANSWER 4 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:419143 BIOSIS
DOCUMENT NUMBER: PREV199900419143
TITLE: Molecular analysis of the role of streptococcal pyrogenic exotoxin A (SPEA) in invasive soft-tissue infection resulting from Streptococcus pyogenes.
AUTHOR(S): Sriskandan, Shiranee (1); Unnikrishnan, Meera; Krausz, Thomas; Cohen, Jonathan
CORPORATE SOURCE: (1) Department of Infectious Diseases, Imperial College School of Medicine at Hammersmith Hospital, Du Cane Road, London, W12 0NN UK
SOURCE: Molecular Microbiology, (Aug., 1999) Vol. 33, No. 4,

pp. 778-790.
ISSN: 0950-382X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Epidemiological studies strongly implicate the bacterial superantigen, streptococcal pyrogenic exotoxin A (SPEA), in the pathogenesis of necrotizing soft-tissue infection and toxic shock syndrome resulting from Streptococcus pyogenes. SPEA can act as a superantigen and cellular toxin ex vivo, but its role during invasive streptococcal infection is unclear. We have disrupted the wild-type spea gene in an M1 streptococcal isolate. Supernatants from toxin-negative **mutant** bacteria demonstrated a 50% reduction in pro-mitogenic activity in HLA DQ-positive murine splenocyte culture, and up to 20% reduction in activity in human PBMC culture. **Mutant** and wild-type bacteria were then compared in mouse models of bacteraemia and streptococcal muscle infection. Disruption of spea was not associated with attenuation of virulence in either model. Indeed, a paradoxical increase in **mutant** strain-induced mortality was seen after intravenous infection. Intramuscular infection with the SPEA-negative **mutant** led to increased bacteraemia at 24 h and a reduction in neutrophils at the site of primary muscle infection. Purified SPEA led to a dose-dependent increase in peritoneal neutrophils 6 h after administration. SPEA is not a critical virulence factor in invasive soft-tissue infection or bacteraemia caused by S. pyogenes, and it could have a protective role in murine immunity to pyogenic infection. The role of this toxin may be different in hosts with augmented superantigen responsiveness.

L7 ANSWER 5 OF 20 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-333330 [29] WPIDS
DOC. NO. CPI: C1998-103378
TITLE: New **mutant Streptococcal SPE-A toxins** - useful for, e.g. prevention or treatment of streptococcal infection or toxic shock syndrome.
DERWENT CLASS: B04 D16
INVENTOR(S): OHLENDORF, D; ROGGIANI, M; SCHLIEVERT, P M; STOEHR, J
PATENT ASSIGNEE(S): (MINU) UNIV MINNESOTA
COUNTRY COUNT: 80
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9824911	A2	19980611	(199829)*	EN	94

RW: AT BE CH DE DK EA ES FI FR GB GH GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI
GB GE GH HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV
MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM
TR TT UA UG US UZ VN YU ZW
AU 9876257 A 19980629 (199845)
EP 948624 A2 19991013 (199947) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9824911	A2	WO 1997-US22228	19971205
AU 9876257	A	AU 1998-76257	19971205
EP 948624	A2	EP 1997-949752	19971205
		WO 1997-US22228	19971205

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9876257	A	Based on	WO 9824911
EP 948624	A2	Based on	WO 9824911

PRIORITY APPLN. INFO: US 1996-32930 19961206
AN 1998-333330 [29] WPIDS
AB WO 9824911 A UPAB: 19991122
Mutant Streptococcal SPE-A toxin or its having at least 1 aa change and being nonlethal compared with a protein corresponding to wild type **SPE-A toxin**, is new. Also claimed are: (1) a DNA sequence encoding the **mutant SPE-A toxin**, and (2) a stably transformed host cell comprising a DNA sequence as in (1).

USE - The **mutant SPE-A toxins** are nontoxic and can produce antibodies that neutralise wild type **SPE-A toxin** activity. The **toxins** can be used in vaccines and therapeutics to generate a protective immune response against streptococcal infection (claimed). They can be used to protect against the development of streptococcal toxic shock syndrome (STSS) (claimed). In addition, the toxins can be used for treating animals with symptoms of streptococcal infection or STSS and in methods for stimulating T cell proliferation and in the treatment of cancer. In particular they can be used for treating T cell lymphomas, and ovarian and uterine cancer.
Dwg.0/13

L7 ANSWER 6 OF 20 MEDLINE DUPLICATE 3
Searcher : Shears 308-4994

ACCESSION NUMBER: 1998187946 MEDLINE
DOCUMENT NUMBER: 98187946
TITLE: Reduced virulence of group A streptococcal Tn916 **mutants** that do not produce streptolysin S.
AUTHOR: Betschel S D; Borgia S M; Barg N L; Low D E; De Azavedo J C
CORPORATE SOURCE: Department of Microbiology, Mount Sinai Hospital, and University of Toronto, Ontario, Canada.
SOURCE: INFECTION AND IMMUNITY, (1998 Apr) 66 (4) 1671-9. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199806

AB Streptolysin S (SLS) is a potent cytolytic **toxin** produced by nearly all group **A streptococci** (GAS). SLS-deficient Tn916 insertional **mutants** were generated from two clinical isolates of GAS, MGAS166s and T18Ps (M serotypes 1 and 18, respectively), by transposon **mutagenesis** using Tn916 donor strain Enterococcus faecalis CG110. Representative nonhemolytic transconjugants SBNH5 and SB30-2 each harbored a single Tn916 insertion in identical loci. The insertion in SBNH5 was located in the promoter region of an open reading frame, designated sagA, rendering it transcriptionally inactive. Protease, streptolysin O, and DNase activities and the production of M protein remained the same in the nonhemolytic **mutants** and the wild-type strains, as did the growth rates and exoprotein profiles. Transconjugants were evaluated in an established murine model by injecting the organisms subcutaneously and monitoring the mice for alterations in weight and the development of necrotic lesions. Animals infected with SBNH5, compared to those infected with MGAS166s, gained weight during the first 24 h (+1.15 versus -1.16 g; $P < 0.05$) and had fewer necrotic lesions (0 versus 7; $P = 0.0007$). Animals infected with SB30-2, compared to those infected with T18Ps, also gained weight within the first 24 h (+0.54 versus -0.66 g; $P < 0.05$) and produced fewer necrotic lesions (1 versus 8; $P = 0.001$). Revertants of the **mutants** in which Tn916 had been excised regained the hemolytic phenotype and the virulence profile of the wild-type strains. This study demonstrates that SLS-deficient **mutants** of GAS, belonging to different M serotypes and containing identical Tn916 **mutations**, are markedly less virulent than their isogenic parents.

L7 ANSWER 7 OF 20 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998254136 MEDLINE
DOCUMENT NUMBER: 98254136
TITLE: High-frequency intracellular infection and erythrogenic toxin A expression undergo phase

variation in M1 group A streptococci.

AUTHOR: Cleary P P; McLandsborough L; Ikeda L; Cue D; Krawczak J; Lam H
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis 55126, USA.. cleary@lenti.med.umn.edu

CONTRACT NUMBER: AI34503 (NIAID)
AI07421 (NIAID)
SOURCE: MOLECULAR MICROBIOLOGY, (1998 Apr) 28 (1) 157-67.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY WEEK: 19980904

AB A clonal variant of serotype M1 group A streptococcus, strain 90-131, disseminated to several continents, where it was associated with severe systemic infections and toxic shock. Although this strain harbours the speA gene and is efficiently internalized by human epithelial cells, clinical isolates often fail to express the erythrogenic toxin under laboratory growth conditions. Cultures of strain 90-131 were observed to phase vary between small, dry, compact and larger, more mucoid colonies. The former were shown to be poorly internalized by epithelial cells. Analysis of RNA by Northern hybridization demonstrated that the emm1, hasA and speA genes were weakly transcribed in cultures derived from the small colonies and highly transcribed in those derived from the large colonies. An insertion **mutation** in mga (the multigene activator) downregulated the invasion of epithelial cells and the transcription of emm1 and hasA, but had little impact on the transcription of speA. These are the first data to suggest the existence of a common regulatory circuit linking intracellular invasion, M protein, hyaluronic acid capsule and erythrogenic **toxin** expression by group **A streptococcus** . Moreover, the genetic instability of **toxin** expression exhibited by this serotype may impact on laboratory studies that attempt to associate toxin production with toxic shock.

L7 ANSWER 8 OF 20 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 97342764 MEDLINE
DOCUMENT NUMBER: 97342764

TITLE: Analysis of toxicity of streptococcal pyrogenic exotoxin A **mutants**.

AUTHOR: Roggiani M; Stoehr J A; Leonard B A; Schlievert P M
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis 55455, USA.

CONTRACT NUMBER: HL 36611 (NHLBI)
SOURCE: INFECTION AND IMMUNITY, (1997 Jul) 65 (7) 2868-75.
Journal code: GO7. ISSN: 0019-9567.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199709
ENTRY WEEK: 19970904

AB Streptococcal pyrogenic exotoxin A (SPE A) is secreted by some strains of Streptococcus pyogenes and is strongly associated with streptococcal toxic shock syndrome (STSS), a severe and often fatal illness. SPE A possesses a number of biological properties, some of which are shared with a group of exotoxins of streptococcal and staphylococcal origins, the pyrogenic **toxin** superantigens (PTSAgs). **SPE A**'s most extensively studied property is superantigenicity. Superantigenic activation of T cells and monocytes stimulates the release of cytokines such as tumor necrosis factors alpha and beta, interleukin 1, and gamma interferon. These endogenous mediators are considered to be the primary cause of capillary leak, hypotension, and shock, the most severe manifestations of STSS. However, several studies have suggested that other properties of SPE A, such as ability to greatly enhance host susceptibility to endotoxin and ability to interact directly with endothelial cells, may play substantial roles in the syndrome. In this work we generated single- and double-site **mutations** of SPE A at residues K16, N20, C87, C90, C98, K157, S195, N20/C98, and N20/K157. The **mutant** SPE A's were analyzed in vivo for their lethal activity and in vitro for their superantigenic ability. Our results indicate that SPE A's ability to induce lethality and endotoxin enhancement does not require superantigenicity, and conversely superantigenicity does not necessarily lead to lethality. Thus, these properties and their relative contributions to the onset of hypotension and shock may be separable. Furthermore, evidence is presented that certain **mutant** toxins may be suitable for use as vaccine toxoids.

L7 ANSWER 9 OF 20 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-099936 [09] WPIDS
DOC. NO. CPI: C1997-031916
TITLE: **Mutant SPE-A** **toxin** with at least one amino acid change is substantially non-lethal - used in vaccine composition for treatment of cancer and streptococcal toxic shock syndrome etc..
DERWENT CLASS: B04 D16
INVENTOR(S): OHLENDORF, D; ROGGIANI, M; SCHLIEVERT, P M; STOEHR, J
PATENT ASSIGNEE(S): (MINU) UNIV MINNESOTA
COUNTRY COUNT: 72
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9640930	A1	19961219	(199709)*	EN	102

RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG

W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN

AU 9662782	A	19961230	(199716)	
EP 832241	A1	19980401	(199817)	EN

R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU NL PT SE

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9640930	A1	WO 1996-US10252	19960607
AU 9662782	A	AU 1996-62782	19960607
EP 832241	A1	EP 1996-921589	19960607
		WO 1996-US10252	19960607

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9662782	A	Based on	WO 9640930
EP 832241	A1	Based on	WO 9640930

PRIORITY APPLN. INFO: US 1995-480261 19950607

AN 1997-099936 [09] WPIDS

AB WO 9640930 A UPAB: 19970228

A **mutant SPE-A toxin** or fragment, which has at least one amino acid change and is substantially non-lethal compared with a wild type **SPE-A toxin**, is new. Also claimed are: (1) an expression cassette comprising a DNA sequence encoding the **mutant SPE-A toxin** operably linked to a promoter functional in a host cell; (2) a DNA sequence (I) encoding a **mutant SPE-A toxin**; (3) a stably transformed host cell, pref. a microorganism, comprising the expression cassette of (1), pref. having an ATCC number 69831; (4) a primer for preparing a **mutant** DNA sequence encoding a **mutant SPE-A toxin**; and (5) a vector, pref. a viral vector, comprising the expression cassette of (1).

USE - **Mutant SPE-A toxins** are used to produce vaccines for protecting an animal against wild type **SPE-A toxin** and for treating

cancer and streptococcal toxic shock syndrome (STSS) (claimed). The recombinant host organism is used in the large scale production of the **mutant SPE-A toxin**. The **mutant SPE-A toxin** causes the production of neutralising antibodies which may be used to ameliorate symptoms of STSS, such as fever, hypotension, group A streptococcal infection, myositis, fascitis, and liver damage. The neutralising antibody is pref. administered in conjunction with antibiotic therapy. The **mutant SPE-A toxins** are esp. useful for treating T cell lymphomas, and ovarian and uterine cancer. It is thought that **mutant SPE-A toxins** can be selectively toxic for T cell lymphoma cells.
Dwg.9/9

L7 ANSWER 10 OF 20 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 96183627 MEDLINE
DOCUMENT NUMBER: 96183627
TITLE: Genetic and phenotypic diversity among isolates of Streptococcus pyogenes from invasive infections.
AUTHOR: Chaussee M S; Liu J; Stevens D L; Ferretti J J
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, USA.
CONTRACT NUMBER: AI-19304 (NIAID)
SOURCE: JOURNAL OF INFECTIOUS DISEASES, (1996 Apr) 173 (4) 901-8.
Journal code: IH3. ISSN: 0022-1899.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199607
AB To determine if recent cases of invasive group A streptococcal disease were caused by strains with a unique characteristic, 117 isolates Streptococcus pyogenes from patients with a variety of diseases, including necrotizing fasciitis and toxic shock syndrome, were analyzed. Significant genomic heterogeneity was observed among selected isolates, as determined using pulsed-field gel electrophoresis. The frequency of the bacteriophage-associated **streptococcal** erythrogenic **toxin** genes **A** and C (speA and speC) among the isolates was 44% (49/112) and 34% (38/112), respectively. Forty-three percent of speA-positive isolates produced **streptococcal** erythrogenic **toxin** (**SPE**) **A** in vitro. Seventy-six percent (85/112) of isolates produced SPE B in vitro, and in contrast to SPE A, little variation in the concentration of SPE B in broth culture supernatants was detected. The genetic and phenotypic heterogeneity observed among isolates from recent cases of severe infection does

not support a clonal basis for the resurgence of invasive streptococcal infections.

L7 ANSWER 11 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1995:109835 BIOSIS
DOCUMENT NUMBER: PREV199598124135
TITLE: Staphylococcal and group **A** **streptococcal** pyrogenic **toxin** superantigens associated with toxic shock syndrome and related illnesses.
AUTHOR(S): Schlievert, P. M. (1); Leonard, B. A. B.; Lee, P. K.; Kreiswirth, B. N.; Eisner, W.; Projan, S. J.; Novick, R. P.
CORPORATE SOURCE: (1) Dep. Microbiol., Univ. Minn., Minneapolis, MN USA
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1994) Vol. 26, No. 0, pp. 303-311.
ISSN: 0941-018X.
DOCUMENT TYPE: Article; General Review
LANGUAGE: English

L7 ANSWER 12 OF 20 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 94011332 MEDLINE
DOCUMENT NUMBER: 94011332
TITLE: Identification of hydrogen peroxide as **a** **Streptococcus** pneumoniae **toxin** for rat alveolar epithelial cells.
AUTHOR: Duane P G; Rubins J B; Weisel H R; Janoff E N
CORPORATE SOURCE: Department of Medicine, Minneapolis Veterans Affairs Medical Center, Minnesota.
CONTRACT NUMBER: AI31373 (NIAID)
R29-AI34051 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1993 Oct) 61 (10) 4392-7.
Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199401
AB Streptococcus pneumoniae infections of the lung are associated with significant damage to the alveolar epithelium. Host phagocytes and pneumolysin, a cytolytic toxin of S. pneumoniae, are believed to contribute to this cellular damage, yet experiments in which these elements are absent demonstrate the presence of an additional soluble S. pneumoniae factor that is toxic to alveolar epithelium. We examined the effects of S. pneumoniae-associated alveolar epithelial cell injury by factors other than S. pneumoniae-derived pneumolysin or phagocyte products by exposing cultured rat type II alveolar epithelial cells (RAEC) to S. pneumoniae **mutants** that lacked pneumolysin activity. We found that **mutant**

pneumolysin-deficient strains of S. pneumoniae produced injury to RAEC similar to that produced by the parent strains. A toxin of type 14 S. pneumoniae was distinguished from pneumolysin by physiochemical (i.e., molecular mass and heat stability) and functional (i.e., hemolytic activity and cytotoxic activity) properties and was identified as hydrogen peroxide. All S. pneumoniae strains tested produced hydrogen peroxide, and in many strains hydrogen peroxide production was comparable to that of activated neutrophils. We conclude that S. pneumoniae produces hydrogen peroxide in concentrations that are cytotoxic to RAEC in vitro and that alveolar epithelial damage due to hydrogen peroxide may be involved in the pathogenesis of host cellular injury in pneumococcal pneumonia.

L7 ANSWER 13 OF 20 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 93162794 MEDLINE
DOCUMENT NUMBER: 93162794
TITLE: Staphylococcus aureus toxic shock syndrome **toxin** 1 and **Streptococcus** pyogenes erythrogenic **toxin A** modulate inflammatory mediator release from human neutrophils.
AUTHOR: Hensler T; Koller M; Geoffroy C; Alouf J E; Konig W
CORPORATE SOURCE: Medizinische Mikrobiologie und Immunologie, AG Infektabwehrmechanismen, Ruhr-Universitat Bochum, Germany.
SOURCE: INFECTION AND IMMUNITY, (1993 Mar) 61 (3) 1055-61. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199305
AB We studied the influence of staphylococcal toxic shock syndrome **toxin** 1 and **streptococcal** erythrogenic (pyrogenic) **toxin A** (ETA) on intact and digitonin-permeabilized human **polymorphonuclear** granulocytes (PMNs). As was shown by reversed-phase high-performance liquid chromatography analysis, toxic shock syndrome toxin 1 or ETA alone, in the absence of any additional stimulus, did not induce the generation of the chemoattractant leukotriene B4 (LTB4) from PMNs in a wide range of concentrations. In addition, pretreatment of intact PMNs with either toxin potentiated formyl-methionyl-leucyl-phenylalanine (fMLP)- and washed Staphylococcus aureus cell-induced generation of LTB4 in a time- and dose-dependent manner. This increase included LTB4 as well as its inactive omega-oxidated compounds. Further studies revealed evidence that toxin exposure was accompanied by enhanced cellular receptor expression for fMLP as well as for LTB4. The intrinsic GTPase activity of membrane fractions was modulated by both toxins. Short-term incubation with

ETA increased the GTPase activity of PMNs up to 141%. Inhibitory effects were obtained when GTP-binding protein functions were stimulated with sodium fluoride (NaF). In addition, specific binding of Gpp(NH)p to GTP-binding protein was inhibited by both toxins during the first 10 min of incubation and was restored at later times of incubation. Our data therefore suggest that both toxins significantly affect the signal transduction pathways of human PMNs, which results in immunomodulatory functions.

L7 ANSWER 14 OF 20 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 94001804 MEDLINE
DOCUMENT NUMBER: 94001804
TITLE: **Mutations** affecting MHC class II binding of the superantigen **streptococcal** erythrogenic **toxin A**.
AUTHOR: Hartwig U F; Fleischer B
CORPORATE SOURCE: First Department of Medicine, University of Mainz, Germany..
SOURCE: INTERNATIONAL IMMUNOLOGY, (1993 Aug) 5 (8) 869-75. Journal code: AY5. ISSN: 0953-8178.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
AB Streptococcal pyrogenic exotoxin A (SPEA) is an important pathogenicity factor of group A streptococci. It is a member of the family of 'superantigens' produced by Staphylococcus aureus and Streptococcus pyogenes, and its T lymphocyte stimulating activity is involved in the pathogenesis of certain diseases caused by pyogenic streptococci. In this study we have generated nine **mutant** SPEA molecules by **substituting** amino acids in the regions of homology between different streptococcal and staphylococcal superantigens. An additional **mutant** was created by deletion of the 10 N-terminal amino acids. The **mutants** were expressed as fusion proteins. Several **mutations** led to a loss of function due to a loss of class II-binding activity. Such loss **mutations** did not cluster to a certain region of the SPEA molecule. Rather, even a **substitution** of neighboring amino acids had opposite effects. None of the loss **mutations** affected the binding of neutralizing mAb and all loss **mutants** could be precipitated in Ouchterlony tests by a polyclonal anti-SPEA serum. We conclude that the functional activities of SPEA, and probably of other superantigens as well, cannot be attributed to a defined region of the molecule.

L7 ANSWER 15 OF 20 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 93132383 MEDLINE
DOCUMENT NUMBER: 93132383

TITLE: Geographic and temporal distribution and molecular characterization of two highly pathogenic clones of Streptococcus pyogenes expressing allelic variants of pyrogenic exotoxin A (Scarlet fever toxin).

AUTHOR: Musser J M; Kapur V; Kanjilal S; Shah U; Musher D M; Barg N L; Johnston K H; Schlievert P M; Henrichsen J; Gerlach D; et al

CORPORATE SOURCE: Department of Pathology, Baylor College of Medicine, Houston, TX 77030..

CONTRACT NUMBER: AI-33119 (NIAID)
RR-05425 (NCRR)

SOURCE: JOURNAL OF INFECTIOUS DISEASES, (1993 Feb) 167 (2) 337-46.
Journal code: IH3. ISSN: 0022-1899.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 199304

AB The molecular population genetics and pathogenic potential of North American and European invasive strains of Streptococcus pyogenes were assessed. Isolates from recent invasive infections and from infections in the 1920s and 1930s were characterized for multilocus enzyme genotype and allelic variation in the gene (speA) that encodes **streptococcal** pyrogenic exotoxin (**SPE**) **A** (scarlet fever **toxin**). A subset of strains was studied for allelic variation in genes that encode SPE B and streptokinase. All contemporary strains assigned to electrophoretic types (ETs) 1 and 2 that synthesize SPE A have the speA2 and speA3 allelic variants, respectively, and their relative virulence in two mouse models is similar to that of strains of the same ET and M protein types recovered earlier. In contrast, ET 1 and 2 isolates from disease episodes in the 1920s and 1930s contain the speA1 allele. The data suggest there may be temporal and geographic variation in the occurrence of clone--virulence factor allele combinations, an observation that may in part explain fluctuations in disease frequency, severity, and character.

L7 ANSWER 16 OF 20 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 91281661 EMBASE

DOCUMENT NUMBER: 1991281661

TITLE: Antigenic cross-reactivity and functional inhibition by antibodies to Clostridium difficile **toxin A**, **Streptococcus** mutans glucan-binding protein, and a synthetic peptide.

AUTHOR: Wren B.W.; Russell R.R.B.; Tabaqchali S.

CORPORATE SOURCE: Dept. of Medical Microbiology, St. Bartholomew's Hospital, Medical College, West Smithfield,London EC1A 7BE, United Kingdom

SOURCE: Infection and Immunity, (1991) 59/9 (3151-3155).
ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A 10-amino-acid repeating sequence of the hemagglutinating portion of Clostridium difficile toxin A has been synthesized and used to produce antisera in rabbits. Antipeptide antibody inhibited toxin A-mediated hemagglutination and neutralized cytotoxic activity. Immunoblot analysis with the antipeptide antibody revealed cross-reactivity with native toxin, a recombinant protein containing the toxin A repeats, and a glucan-binding protein from Streptococcus **mutants** whose primary structure has repeating amino acid motifs similar to those of the synthetic peptide. A polyclonal antibody against the glucan-binding protein, which cross-reacted with purified toxin A, also inhibited toxin A-mediated hemagglutination and neutralized cytotoxic activity. We recently identified toxin A and the glucan-binding protein as members of a novel family of clostridial and streptococcal binding proteins based on conserved repeating amino acid motifs at the C-terminal region of the molecules. This study provides immunological and functional evidence of the predicted relationship between toxin A and the glucan-binding protein and further implicates the repeating subunits as ligand-binding domains in this family of proteins.

L7 ANSWER 17 OF 20 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 1991:502653 BIOSIS
DOCUMENT NUMBER: BA92:125613
TITLE: BACTERIAL TOXINS INDUCE HEAT SHOCK PROTEINS IN HUMAN NEUTROPHILS.
AUTHOR(S): HENSLER T; KOELLER M; ALOUF J E; KOENIG W
CORPORATE SOURCE: LEHRSTUHL MED. MIKROBIOLOGIE IMMUNOLOGIE, ARBEITSGRUPPE INFEKTABWEHRMECHANISMEN, RUHR-UNIVERSITAET BOCHUM, UNIVERSITAETSSTRASSE 150, D-4630 BOCHUM 1, FRG.
SOURCE: BIOCHEM BIOPHYS RES COMMUN, (1991) 179 (2), 872-879.
CODEN: BBRCA9. ISSN: 0006-291X.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB We studied the influence of different bacterial toxins (alveolysin; toxic shock syndrome toxin 1, TSST-1 and erythrogenic toxin A, ETA) on the expression of heat shock proteins (hsps) in isolated human **polymorphonuclear** granulocytes (PMNs). As was shown by Western blotting (anti-hsp72) ETA and TSST-1 were potent inducers of hsps at low toxin concentrations (10 ng/ml). Alveolysin led to the expression of hsps at hemolytic concentrations (1 HU; 700 ng/ml) whereas at subhemolytic concentrations (7 ng/ml) no heat shock